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Original Research Article

ASSESSMENT OF CONTROVERSIAL RISK FACTORS IN DEVELOPMENT OF BREAST CANCER: A STUDY FROM LOCAL POPULATION

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Abstract: *Breast cancer is the most common cause of death worldwide in women. Several predisposing risk factors have been identified making its incidence constantly rising. The aim of the current study was to analyze thyroid hormone, vitamin D, and 8-hydroxydeoxyguanosine (8-OHdG) as risk factors in the onset of breast cancer. In the present case and control study, a total of two hundred and seventy-four (n=274) participants were included after taken the informed consent individually. The participants were further stratified into two groups. Group A consisted of one hundred and thirty-seven age and sex-matched healthy individuals presented at OPD whereas group B had one hundred and thirty-seven diagnosed cases of breast cancer. Serum vitamin D, thyroid hormone, and 8-OHdG were measured by commercially available ELISA kits by using the blood samples drawn from the study population. Data were analyzed with SPSS V21.0 and a p-value less than 0.05 considered statistically significant. Cases and control were comparable in terms of age, BMI, and gender distribution. Vitamin D deficiency was present in 78.4% of cases and 63.5% of control. (OR 3.83 95% CI 1.38 -10.5) 8-OHdG was present in 56.8% and 11.1% in cases and control respectively (P 0.001). Similarly, MDA was found positive in 67.6% of cases and 21.6% of control (P 0.001). Vitamin D and thyroid hormone deficiency and elevated levels of reactive oxygen species (ROS) are supposed to associate with increased risk in the development of breast cancer.*

Keywords: Breast Cancer, Vitamin D deficiency, Reactive Oxygen Species, 8-hydroxydeoxyguanosine, thyroid hormone

Introduction

Breast cancer is a malignant tumor that has developed from undifferentiated cells of the breast tissue. This tissue can develop rare benign conditions known as angiosarcomas or phyllodes tumors. In most of the cases breast tumors are malignant and spread to the other tissues such as bones (Zülch, 2013). Breast cancer is the most commonly occurring cancer in women, comprising almost one third of all malignancies in females (Feng *et al*., 2018). According to World Health organization (WHO), breast cancer is diagnosed in 1.5 million women every year and the incidence rate is on rise globally (Silva *et al*., 2019). In USA, the most common cancer in women is breast cancer (excluding skin cancers) and it is 2nd leading cause of mortality among women after lung cancer. In 2016, 124 new female breast cancer cases were reported per 100,000 women and 20/100,000 die because of it (Pellegriti *et al*., 2013). Pakistan has highest age standardized incidence rate (ASIR) of breast cancer, affecting 23% of females (DeSantis *et al*., 2017). Estimated prevalence in 2012 was 119710, causing mortality of 16,232 patients (Begum, 2018). Age, reproductive factors, personal or family history of breast disease, genetic pre-disposition and environmental factors have been associated with an increased risk for the development of female breast cancer (Sarwar and Saqib, 2017). At presentation lump in breast is painless and nipple retraction of occasionally with breast skin changes. The need of the day is to recognize the causative factors and sort out the ways to minimize them. Oxidative stress is the emerging risk factor not only in breast cancer but also proved to be contributory factor in almost all types of cancers. Oxidative stress can be broadly defined as an imbalance between oxidants and antioxidants in favor of the oxidants, potentially leading to damage. If the levels of reactive

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oxygen species are high and overcome the antioxidant defense mechanisms of the human body, oxidative damage can occur to lipids, proteins, or directly to DNA. Reactive Oxygen Species are produced by both enzymatic and non-enzymatic systems within eukaryotic cells and play important roles in cellular physiology and pathophysiology (Majeed *et al*., 2014). Although physiological concentrations are crucial for ensuring cell survival, ROS overproduction is detrimental to cells, and considered as key-factors for the development of several diseases, such as neurodegenerative diseases, cardiovascular disorders, and cancer. Cancer cells are usually submitted to higher ROS levels that further stimulate malignant phenotype through stimulus to sustained proliferation, death evasion, angiogenesis, invasiveness, and metastasis (Sun *et al*., 2017). Breast cancer cells have been shown to be susceptible to oxidative damage and have high levels of oxidative stress, including protein damage, DNA damage, and lipid peroxidation (Qi *et al*., 2015). However, less attention has been given to the development of redox system-targeted strategies for breast cancer therapy. Other known factors are vitamin D and Thyroid hormones. Thou the formation and release of thyroid hormone is depended on the normal functioning of vitamin D but thyroid hormone is considered individually a risk factor of breast cancer. 1, 25-dihydroxyvitamin (cholecalciferol) or Vitamin D is fat-soluble vitamin essential for calcium hemostasis and bone development. Primary source of vitamin D is through dermal synthesis by ultraviolet rays in sunlight. Subclinical deficiency exits in many people which are a risk factor for osteoporosis, bone fractures, cardiovascular disease, cancer and diabetes (Wu *et al*., 2016). It possesses anti-carcinogenic properties which include cell proliferation inhibition, invasion, metastasis and angiogenesis and induction of apoptosis and differentiation (Lee *et al*., 2017). In recent developments, a vitamin D receptor (VDR) gene has been reported that increase risk of breast cancer. Efforts have been directed toward identification of vitamin D deficiency as its levels (levels ≥45 ng/mL) may protect against breast cancer (Parva *et al*., 2018). The association between thyroid disorders and breast cancer has been studied in the past with mixed results. Most of the articles published to date have relied on studies of relatively small sample sizes (Hernando *et al*., 2020; Kleine *et al*., 2019; Wolf *et al*., 2020). A recent systematic review and meta-analysis that included 13 population-based studies with 24,808 participants through June 2016 found that neither hypothyroidism nor hyperthyroidism was related to the risk of breast cancer (Kuijpens *et al*., 2005). A study utilizing the national registry in Denmark by Sogaard and his colleagues also examined this association without age stratification and found a similar result for hyperthyroidism, but not for hypothyroidism (Søgaard *et al*., 2016). The current study assessed the association of reactive oxygen species, vitamin D and thyroid profile in development of breast cancer.

Material and methods

The current case and control study included two hundred and seventy-four participants divided into two groups. Group A was the control group and had one hundred thirty-seven age and sex matched healthy participants that visited the OPD of different Hospitals of the Lahore. Group B constituted the one hundred and thirty-seven diagnosed cases of breast cancer. All participants were included in the study after taken the informed consent and the approval of ethical review board of Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore. Data was collected on specially design Proforma and 5ml of blood sample was withdraw and stored for further analyses from different hospitals of Lahore (31.5204° N; 74.3587° E). Commercially available ELISA kits were used to analyzed the different variables such as vitamin D, thyroid hormone and Reactive oxygen species. A method has already been used for screening lipid peroxidation product (MDA) by Thiobarbituric acid reactive substances with the help of spectrophotometer. In this method we took 200 µl of sample, then 0.2ml Sodium dodecyl sulfate was added, then 20% of acetic acid by 1.5 ml was added, and lastly TBA was added about 1.5ml. After this, the solution was centrifuged at 3000 rpm for 10 min. Two layers were formed after centrifugation and upper organic layer was taken from test tube to check absorbance at 532 nm. A sample size of 274 patients (1:1 ratio, 137 Cases and 137 Control) is calculated by using WHO sample size calculator with 80% power of test and 5% level of significance. All data was recorded in proforma and analyzed with SPSS version 20. Qualitative data in cases and controls were presented in frequency and percentages. Quantitative data such as age and level of vitamin D was presented in mean and standard deviation. Odd Ratio were calculated between cases and control. Chi Square test and student t test/ ANNOVA were used to compare categorical and numerical data respectively. *P* value < 0.05 considered significant.

Results

Mean age of patents was 52.2 ± 9.1 years and 53.7 ± 10.9 years in case and control group respectively.

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Majority of patients in both groups were > 50 years. (Table 1)

Table 1: Age distribution of patients

AGE DISTRIBUTION OF PATIENTS				
Age	**Cases**		**Control**	
	n	**%**	**n**	**%**
Age < 50 Years	64	37.8	67	45.9
Age > 50 Years	73	62.2	80	54.1
Total	137	100.0	137	100.0
Mean + SD	52.2 ± 9.1		53.7 ± 10.9	

Cases and control were comparable in terms of height, weight and BMI as *p* valve is insignificant (Table 2)

Table 2: Height, weight and BMI

HEIGHT, WEIGHT & BMI					
Parameter	**Cases**		**Control**		**P Value**
	Mean	**SD**	**Mean**	**SD**	
Height (cm)	153.1	6.3	155.1	4.7	0.219
Weight (Kg)	58.24	7.4	56.9	7.2	0.420
BMI (Kg/m2)	37.9	4.9	36.6	4.4	0.226

Vitamin D deficiency was present in 78.4% of cases and 63.5% of control. (OR 3.83, CI 95) (Table 3).

Table 3: Assessment of vitamin d levels and risk of development of breast cancer

Vitamin D Deficiency		
Vitamin D n (%)	**Breast Cancer**	**No Breast Cancer**
	Cases	**Control**
Deficiency (YES)	79 (78.4)	68 (63.5)
Deficiency (NO)	58 (21.6)	69 (36.5)
Total	137 (100.0%)	137 (100.0)
Odd ratio		**3.83**

ROS namely 8OHdG and MDA were analyzed in cases and control. Elevated levels of 8-OHdG were present in 56.8% and 11.1% in cases and control respectively (P 0.001). Similarly, MDA was found positive in 67.6% case and 21.6% of control. (*P*= 0.001)

(Table 4)

Table 4: Assessment of levels of ROS and risk of development of breast cancer

REACTIVE OXYGEN SPECIES (ROS)					
8OHdG	**Cases**		**Control**		***P*-Value**
	n	**%**	**n**	**%**	
ELEVATED (YES)	71	56.8	53	11.1	**0.001**
ELEVATED (NO)	66	43.2	84	91.9	
Total	137	100.0	137	100.0	
MDA	**Cases**		**Control**		***P*-Value**
	n	**%**	**n**	**%**	
ELEVATED (YES)	75	67.6	58	21.6	**0.001**
ELEVATED (NO)	62	32.4	79	78.4	
Total	137	100.0	137	100.0	

Thyroid levels as estimated free T3 and free T4 were statistically low in the group B as compare to the Group A (*p*=0.041, p=0.035 respectively) (Table-5)

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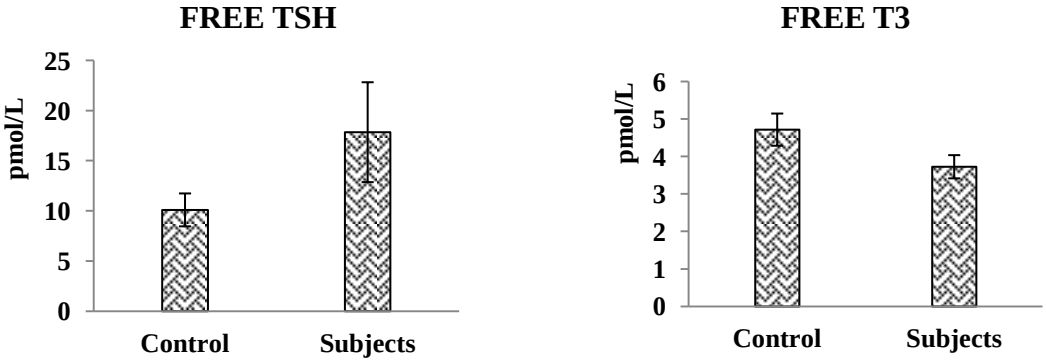
Table 5: Estimation of thyroid profile in breast cancer patients

Variables	Control	Patient	*P*-Value
FT3 pmol/L	4.72±0.24	3.73±0.189	0.041
TSH pmol/L	10.11± 1.64	17.86±4.98	0.035

Figure-1: Levels of free T3 and TSH in both groups



Discussion

Breast cancer has been considered as the most common type of cancer among the women. Known and well-established risk factors for breast cancer include age, family history, and the density of breast tissue, parity, overweight, alcohol intake, and genetic risk factors such as BRCA mutations (Tseng *et al*., 2015). Recently, vitamin D receptor (VDR) genes were reported to increase breast cancer risk. Several molecular breast cancer subtypes have been identified: luminal A and B (accounting for 50%-60% of breast cancer cases), basal-like or triple-negative (10%-20% of breast cancer cases) and human epidermal growth factor receptor 2 (HER2)-enriched (10%-15% of cases) (Krashin *et al*., 2019; Weng *et al*., 2018). Vitamin D receptor genes operated by vitamin D have important roles in the mammary gland through regulation of calcium transport during lactation, hormone differentiation, and milk production. Many efforts and enormous research have been directed toward identifying vitamin D as a breast cancer risk factor to be targeted for cancer prevention. This is because circulating vitamin D levels (levels ≥45 ng/mL) may protect against breast cancer (Hernando *et al*., 2020; Parva *et al*., 2018) and because breast cancer chemoprevention drugs that alternate the carcinogenesis process such as estrogen receptor modulators, tamoxifen, raloxifene, and aromatase inhibitor have high toxicities and not effective in the aggressive estrogen receptor–negative (ER−) breast cancers (Journy *et al*., 2017; Kitahara *et al*., 2019). Our study shows that vitamin D was found deficient in 78.4% case and 63.5% of control. (OR 3.83 95% CI 1.38 -10.5) Our result were comparable with Shaukat *et al* where serum vitamin D levels were significantly lower in cases (85.7%) than controls (55.8%). ORs (95% CIs) for breast cancer risk were 7.8 (1.99-30.58) for women with vitamin D concentrations less than 20 ng/mL. Bilinski *et al* examined the association between vitamin D status and risk of breast cancer in Australian women and they reported that 25(OH) D concentration below 75 nmol/L at diagnosis was associated with a significantly higher risk of breast cancer (Dong *et al*., 2018). Compared with subjects with sufficient 25(OH) D concentration, the odds ratios of breast cancer were 2.3 (95% CI = 1.3-4.3), 2.5 (95% CI = 1.6-3.9) and 2.5 (95% CI = 1.6-3.8) for subjects categorized as severely deficient, deficient, or insufficient vitamin D status, respectively (Hall *et al*., 2008; Wang *et al*., 2018). Park *et al* studied the association between vitamin D and breast cancer risk among the Asian population. Examined the association between serum 25(OH) D and breast cancer risk stratified by menopausal status and hormone receptor (HR) status of the tumor. Women with vitamin D deficiency had 27% increased the risk for breast cancer compared with women who have sufficient levels of serum 25(OH) D. This association did not significantly vary by menopausal status. HR status has significant inverse association and this association was more pronounced in HR-negative breast cancer, particularly with patients with triple-negative breast cancer (Davis *et al*., 2009; Joo and Jetten, 2010). Few studies have shown no association between 25(OH) D deficiency and risk breast cancer. Eliassen *et al* investigated whether plasma 25(OH) D interacts with breast tumor expression of VDR and its risk of breast cancer in women followed more than

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20 years. No overall association was observed between plasma 25(OH) D and breast cancer risk. Women with high plasma 25(OH) D levels in the summer have a reduced breast cancer risk. Plasma 25(OH) D may be inversely associated with risk of tumors expressing high levels of VDR (Tosovic *et al*., 2012). Neuhouser *et al* in multivariate-adjusted models for colorectal cancer, the association strengthened (OR = 4.45, 95% CI = 1.96-10.10), whereas in multivariate-adjusted breast cancer models, associations were not significant (OR = 1.06, 95% CI = 0.78, 1.43) (Glover *et al*., 2015; Khan *et al*., 2016).

Our study has shown significant association with ROS and breast cancer. ROS were more frequent in cases than control (*P*=0.001). Sarmiento-Salinas *et al* has shown triple negative breast cancer (TBNC) have been found to have increased ROS levels, our data shows increased ROS levels in all the TNBC cell lines studied in comparison to an ER+ breast cancer cell line or the non-tumorigenic cells (Chi *et al*., 2012). Other cancer types that have also been characterized by increased oxidation levels and in which a role of ROS has been proposed in the promotion of malignancy include prostate, gastric, and pancreatic cancer (Lin *et al*., 2013). In the literature, there is conflicting evidence regarding the use of antioxidants during cancer progression and treatment. In normal cells or pre-cancerous lesions, ROS have been proposed to induce DNA damage and increase oncogenic mutations, raising the possibility that dietary supplementation with antioxidants could suppress the initiation or progression of some types of cancer. However, antioxidant treatment is known to suppress cancer initiation in some contexts and increase cancer progression in others. Moreover, the use of dietary antioxidants has not been shown to reduce cancer incidence and in fact, antioxidant supplementation has actually increased incidence and death from some types of cancer including lung cancer or increase the risk of developing another type of unrelated diseases (Wang and Chen, 2013).

Clinical studies have found a protective association between hypothyroidism and breast cancer development; this may be due to the biological effect of T3 at the cellular level, the interaction of T3 with TRs, or modulation of the thyrotropin receptor (TSH-R) (Lin *et al*., 2015). The antioxidant property of iodine may also play a role, especially considering the capacity of breast tissue to transport and concentrate iodide (Chi *et al*., 2016; Wu *et al*., 2013). To date, there is still limited epidemiologic evidence for the link between thyroid replacement treatment and the risk of breast cancer. In this study, we found an inverse association between hypothyroidism and invasive breast cancer development among women (*p*=0.043,0.035). Weng and his colleagues using the Taiwanese national database that included 103,466 Asian women, found that women younger than 55 years with a history of hyperthyroidism had a 16% higher risk of developing breast cancer compared with those without a history of thyroid disorder (Weng *et al*., 2018). A history of hypothyroidism was also found to be associated with a 19% higher risk, without stratification by age (Chen *et al*., 2013; Kaminskyy *et al*., 2013).

Conclusion

In our study age, BMI and education status did not show any statistically significant association with breast cancer risk. In addition, our study showed no significant association of BMI and vitamin D deficiency. The topic of vitamin D deficiency and breast cancer risk is a field of intense study and many aspects of it require further investigations.

Conflict of interest

The authors declared absence of any conflict of interest.

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